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PATENT
Attorney Docket No. INL-054DV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Dahlbäck et al.		
SERIAL NO.:	10/037,296	GROUP NO.:	1644
FILING DATE:	December 21, 2001	EXAMINER:	Not yet assigned
TITLE:	METHODS AND REAGENTS FOR DETERMINING PROTEIN S		

Box Missing Parts
Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Please make the following amendments prior to examination.

In the Specification

On page 1, after the Title, please insert the following paragraph:

--Related Applications

This is a divisional of U.S. Application Serial No. 08/978,285, which was filed November 5, 1997, and claims the benefit of U.S. Provisional Application Serial No. 60/035,363, which was filed January 10, 1997 and Swedish Patent Application No. 9604378-1, which was issued November 27, 1996, the disclosures of which are incorporated by reference herein.--

On page 1, after the Related Applications, please insert the following section title:

--**Summary of the Invention**--

On pages 3-4 of the specification, please delete the paragraph beginning at line 21 and replace with:

--Moreover, assays for free protein S based on immobilized monoclonal antibodies directed to free protein S, which are used as immobilized antibody in standard ELISA (Enzyme

Linked Immuno Sorbent Assay) to capture free protein S in plasma, have been described in the literature and are also commercially available from Stago (Amiral et al., Blood Coag: Fibrinol. 1994, 5:179-186, and Wolf et al., Blood Coag. Fibrinol. 1994, 5:187-192). In such tests, plasma dilutions in buffer containing calcium are incubated in microtitre plates containing monoclonal antibodies specific for free protein S, and, subsequent to washing steps, protein S bound to the bound to the monoclonal antibodies can be detected with the use of a second mono- or polyclonal antibody directed to protein S. However, such assay are extremely expensive. Furthermore, the antibodies used in these tests are not well characterized and they have not been raised specifically against any region of protein S suggested to be involved in the binding of C4BP to protein S. Rather, these antibodies have been raised against the entire protein S molecule, whereafter antibodies having specificity for free protein S have been selected.--

On page 22 of the specification, please delete Table 1 and replace with:

--Table 1. Synthetic peptides

Designation	**Amino acid residue sequence**	**hPS SEQ ID NO**
BD4	LDGCIRSWNLMKQGASGIKEIIQEKQNKHCLVT	405-437 (SEQ ID NO:1)
BD6	YNGCMEVNINGVQLDLDEAISKHNDIRAHSCPSV	595-628 (SEQ ID NO:2)
SL1	KPENGLLETKVYFAGFPRK	374-392 (SEQ ID NO:3)
SL2	EKGSYYPGSGIAQFHIDYNNVS	439-460 (SEQ ID NO:4)
SL3	SDQQSHLEFRVNNLEKSTPLK	527-550 (SEQ ID NO:5)
SL4	DKAMKAKVATYLGGLPDVPFSAT	567-589 (SEQ ID NO:6)
SL5	LVTVEKGSYYPGSGIAQ	435-451 (Residues 1-17 of SEQ ID NO:7)
SL6	SGIAQFHIDYNNVSSAEGWHVN	447-468 (Residues 13-34 of SEQ ID NO:7)
SL7	LVTVEKGSYYPGSGIAQFHIDYNNVSSAEGWHVN	435-468 (SEQ ID NO:7)--

On page 29 of the specification, please delete the Abstract and replace with:

--Abstract

The present invention relates to an assay for free protein S comprising the addition of a ligand specific for free protein S to a biological fluid sample to form a protein S/ligand complex,

and subsequent determination of the amount of the complex formed in the sample. The ligand specific for free protein S is comprised of the C4b binding protein (C4BP), or part thereof, or a compound comprising an amino acid residue sequence that binds specifically to the binding site for C4BP in protein S. The present invention further relates to antibodies specific for free protein S, which can be used as ligands in the assay of the invention, and with protein S related polypeptides, which can be used to produce such antibodies. In addition, the present invention is related to diagnostic test systems, suitable in kit form, comprising the present ligand and at least one further reagent required in the assay for free protein S.--

Applicants believe that no additional fees are necessitated by this Preliminary Amendment. However, in the event that any additional fees are due, the Commissioner is hereby authorized to charge Deposit Account No. 20-0531.

Respectfully submitted,

Date: June 3, 2002
Reg. No. 43,153
Tel. No. (617) 248-7103
Fax No. (617) 248-7100

Diana M. Steel
Attorney for Applicants
Testa, Hurwitz & Thibeault, LLP
High Street Tower
125 High Street
Boston, MA 02110

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MARKED-UP VERSION OF THE AMENDMENTS TO THE SPECIFICATION

On pages 3 of the specification, the second paragraph is deleted and replaced with:

Moreover, assays for free protein S based on immobilized monoclonal antibodies directed to free protein S, which are used as immobilized antibody in standard ELISA (Enzyme Linked Immuno Sorbent Assay) to capture free protein S in plasma, have been described in the literature and are also commercially available from Stago (Amiral et al., Blood Coag: Fibrinol. 1994, 5:179-186, and Wolf et al., Blood Coag. Fibrinol. 1994, 5:187-192). In such tests, plasma dilutions in buffer containing calcium are incubated in [micrometer] microtiter plates containing monoclonal antibodies specific for free protein S, and, subsequent to washing steps, protein S bound to the bound to the monoclonal antibodies can be detected with the use of a second mono- or polyclonal antibody directed to protein S. However, such assay are extremely expensive. Furthermore, the antibodies used in these tests are not well characterized and they have not been raised specifically against any region of protein S suggested to be involved in the binding of C4BP to protein S. Rather, these antibodies have been raised against the entire protein S molecule, whereafter antibodies having specificity for free protein S have been selected.

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SL7	LVTVEKGSYYPGSGIAQFHIDYNNVSSAEGWHV N	435-468 (SEQ ID NO:7)--

On page 29 of the specification, the Abstract is deleted and replaced with:

The present invention [is concerned with] relates to an assay for free protein S comprising the addition of a ligand specific for free protein S to a biological fluid sample to form a protein S/ligand complex, and subsequent determination of the amount of [said] the complex formed in the sample. The ligand specific for free protein S is comprised of the C4b binding protein (C4BP), or part thereof, or a compound comprising an amino acid residue sequence that binds specifically to the binding site for C4BP in protein S. The present invention [is] further [concerned with] relates to antibodies specific for free protein S, which can be used as ligands in the assay of the invention, and with protein S related polypeptides, which can be used to produce such antibodies. In addition, the present invention is related to diagnostic test systems, suitable in kit form, comprising the present ligand and at least one further reagent required in the assay for free protein S.

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